Copy for the Elected Office (EO/US)

PCT/GB97/02477

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

FISHER, Adrian, John
Carpmaels & Ransford
43 Bloomsbury Square
London WC1A 2RA
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
09 September 1998 (09.09.98)

Applicant's or agent's file reference

IMPORTANT NOTIFICATION

International application No.
PCT/GB97/02477

International filing date (day/month/year)
10 September 1997 (10.09.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
JOHNSON & JOHNSON MEDICAL, INC.
2500 Arbrook Boulevard
Arlington, TX 76004-3030
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
JOHNSON & JOHNSON MEDICAL LIMITED
Erskine House
68-73 Queen Street
Edinburgh EH2 4NH
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

002227416

FC1

PCT/GB97/02477

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 April 1998 (27.04.98)	
International application No. PCT/GB97/02477	**Applicant's or agent's file reference**
International filing date (day/month/year) 10 September 1997 (10.09.97)	**Priority date** (day/month/year) 11 September 1996 (11.09.96)
Applicant GRADY, Michael, William et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 April 1998 (07.04.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer A. Addae-Ruesch
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
JJM-0399		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB97/02477	10/09/1997	11/09/1996
International Patent Classification (IPC) or national classification and IPC		
C08B15/00		
Applicant		
JOHNSON & JOHNSON MEDICAL, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/04/1998	15.12.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Contet, F Telephone No. (+49-89) 2399-8671

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB97/02477

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-11 as received on 28/09/1998 with letter of 25/08/1998

Drawings, sheets:

1/2,2/2 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 12 - 16
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 -11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1 - 11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1 - 11
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document :

D : EP-A-0140 596

I- Article 34 (2) b) PCT :

The present claims 1 to 11 satisfy the requirements of said Article.

II- Novelty and Inventive step :

Document D, which is considered to represent the most relevant state of the art, discloses a protein/polyanionic polysaccharide complexes.
The polysaccharide is selected from alginate, cellulose such as carboxymethyl cellulose, sulfate dextrans or gum such as carrageenans or xanthan gum (page 4, line 32 - 34) and the protein is preferably collagen.
Said complex is preferably stabilised with a multivalent cation such as calcium (page 5, lines 8 to 26).
The complex such obtained is used for preparing burn or wound dressing or a surgical implant (Claims 18 to 22, page 2, lines 1 to 8 and Ex. 4 and 5).

However the specific use of a sulfated polysaccharide for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase, especially a chronic wound, was neither known nor suggested from the available prior art.

Thus novelty and inventive step of the claimed subject-matter can be acknowledged.

III- Industrial applicability

Preparation of a composition for the treatment of a chronic wound.

Re Item VIII

Certain observations on the international application

As required by Rule 5.1(a)(iii) PCT, the description is not in conformity with the claims, on the basis of which this report has been drafted.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 97/ 02477	10/09/1997	11/09/1996
Applicant		
JOHNSON & JOHNSON MEDICAL, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 1
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

Inter[redacted]l Application No
PCT/GB 97/02477

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B15/00 C08B37/04 A61K31/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHARD G. SCHWEIGER ET AL.: "Polysaccharide Sulfates." CARBOHYDRATE RESEARCH., vol. 21, 1972, AMSTERDAM NL, pages 275-281, XP002049669 see the whole document ---	1-16
X	KURT MAURER ET AL.: "Die Synthese heparinwirksamer Verbindungen aus carboxylierter Cellulose." CHEMISCHE BERICHTE., vol. 80, no. 1, 1947, WEINHEIM DE, pages 179-187, XP002049670 see page 181 ---	1-16
X	FR 1 056 772 A (HENRI MORREN) 2 March 1954 see page 1, column 2, last paragraph - page 2, column 1, paragraph SECOND --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 1997	14/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lensen, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte[redacted]al Application No
PCT/GB 97/02477

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 140 596 A (JOHNSON & JOHNSON) 8 May 1985 see page 4, line 21 - page 5, line 4	1-16
A	US 3 709 877 A (DEGER TUNE) 9 January 1973	
A	DE 25 46 699 A (SUMITOMO CHEMICAL) 29 April 1976	
A	US 4 879 282 A (SALIBA JR.) 7 November 1989	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte[redacted]al Application No
PCT/GB 97/02477

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 1056772 A	02-03-54	NONE	
EP 140596 A	08-05-85	GB 2148901 A	05-06-85
		AU 573626 B	16-06-88
		AU 3380384 A	18-04-85
		CA 1234102 A	15-03-88
		DE 3472263 A	28-07-88
		US 4614794 A	30-09-86
US 3709877 A	09-01-73	AR 192687 A	28-02-73
		AU 4990672 A	13-06-74
		CA 965783 A	08-04-75
		GB 1407064 A	24-09-75
		ZA 7208413 A	26-06-74
DE 2546699 A	29-04-76	JP 51052484 A	10-05-76
		FR 2287911 A	14-05-76
US 4879282 A	07-11-89	AU 1573588 A	10-10-88
		CA 1315683 A	06-04-93
		WO 8806840 A	22-09-88
		US 5037810 A	06-08-91

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C08B 15/00, 37/04, A61K 31/715

A1

(11) International Publication Number: **WO 98/11141**

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/GB97/02477

(22) International Filing Date: 10 September 1997 (10.09.97)

(30) Priority Data:
9618958.4 11 September 1996 (11.09.96) GB

(71) Applicant *(for all designated States except US)*: JOHNSON & JOHNSON MEDICAL, INC. [US/US]; 2500 Arbrook Boulevard, Arlington, TX 76004-3030 (US).

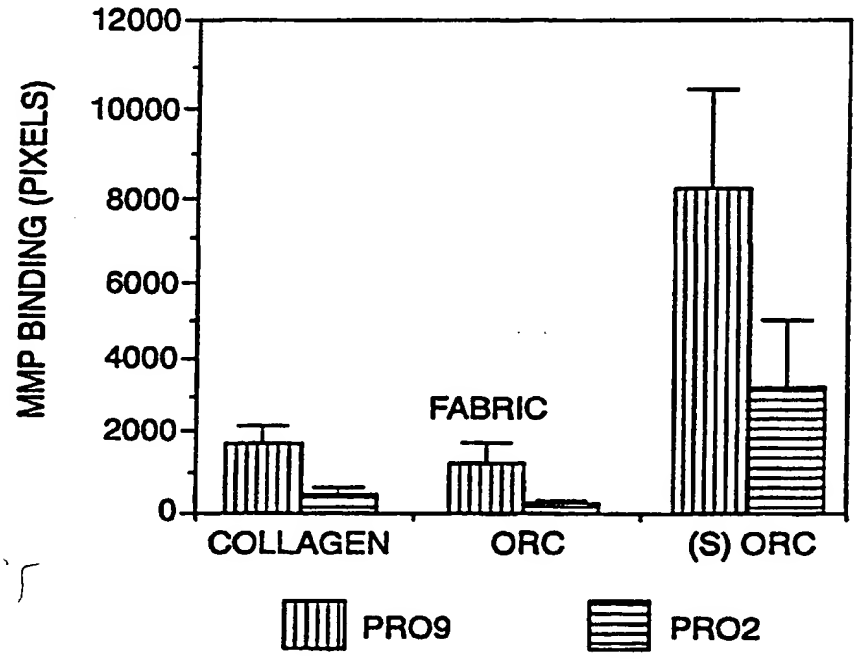
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GRADY, Michael, William [GB/GB]; 4 Cornmill, Menston, West Yorkshire LS29 3RX (GB). DOYLE, Peter, John [GB/GB]; 64 Brooklands Way, Menston, West Yorkshie LS29 6PP (GB). SINCLAIR, Laura [GB/GB]; 29a Church Street, Ilkley, West Yorkshire LS29 9DR (GB). HOUSTON, Paul [GB/GB]; 111 Glasgow Road, Edinburgh EH12 8NP (GB).

(74) Agent: FISHER, Adrian, John; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT



(57) Abstract

The invention provides sulfated oxidised regenerated cellulose compounds, sulfated alginates, and the salts and hydrates thereof. The compounds are obtained by sulfation of oxidised regenerated cellulose with sulfur trioxide. The invention also provides pharmaceutical compositions comprising the compounds, in particular compositions for the treatment of medical conditions mediated by a matrix metalloproteinase and anticoagulant pharmaceutical compositions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

SULFATED POLYSACCHARIDES AND USES THEREOF IN MEDICAL TREATMENT

The present invention relates to novel sulfated polysaccharides obtained by sulfation of cellulose derivatives or polyanionic polysaccharides and uses thereof in pharmaceutical and wound treatment compositions.

Sulfated saccharide and polysaccharide derivatives, in which one or more hydroxyl groups on individual saccharide residues are replaced by sulfate groups, are known. Such sulfated saccharides can occur naturally, as in the case of the sulfated glucosaminoglycans such as heparan sulfate and chondroitin sulfate. Other sulfated saccharides, oligosaccharides and polysaccharides have been prepared by sulfation of naturally occurring saccharides, oligosaccharides or polysaccharides.

A known synthetic sulfated saccharide derivative is sucrose octasulfate, obtained by sulfation of sucrose. The insoluble aluminum salt of sucrose octasulfate (known as sucralfate) has been proposed for a number of medicinal uses, including the enhancement of wound healing in EP-A-0230023.

US-A-2697093 describes the sulfation of cellulose, inulin, starch or dextrin with sulfur trioxide-tertiary amine reagents. The resulting sulfated materials are stated to be useful as thickeners for paste, adhesives, and additives for muds used in drilling oil wells.

Certain sulfated polysaccharides, and in particular the sulfated glucosaminoglycans, are used in medicine as anticoagulants. In addition, heparin, a highly polydisperse copolymer of 1-4 linked glucosamine and uronic acid residues, plays an important role in the control of blood coagulation. Heparin binds the serine protease inhibitor antithrombin producing a complex which accelerates the proteolysis of the enzyme responsible for coagulation. The

anticoagulant properties of heparin and its analogs have been known for some time and have been usefully applied in the biomedical field. The preparation of heparin and sulfated glucosaminoglycans from natural sources is expensive, and therefore an alternative is desirable.

It has now been found that sulfation of cellulose derivatives such as oxidised regenerated cellulose (ORC) or polyanionic polysaccharides such as sodium alginate results in novel sulfated polysaccharide materials that provide unexpected advantages as wound healing materials, and further exhibit anticoagulant properties.

Accordingly, the present invention provides a sulfated polysaccharide selected from a sulfated cellulose derivative and a sulfated polyanionic polysaccharide.

The term "cellulose derivative" encompasses any biologically acceptable ester, ether, hydrolysis product or oxidation product of cellulose. Preferred cellulose derivatives include carboxymethyl cellulose, hydroxyethyl cellulose, cellulose acetate and, especially, oxidized cellulose derivatives such as oxidized regenerated cellulose (ORC), or the oxidized cellulose ethers and esters described and claimed in the provisional US patent application USSN 60/020,758 assigned to the same assignee as the present application and filed on 28th June 1996 and entitled "Bioresorbable Medical Devices from Oxidized Cellulose Derivatives". Preferred materials are obtained by oxidation of hydroxyethyl cellulose, carboxymethyl cellulose or cellulose acetate with dinitrogen tetroxide. The most preferred material is oxidized regenerated cellulose (ORC).

The term "polyanionic polysaccharide" encompasses any biologically acceptable polysaccharide in which a plurality of the saccharide residues in each polysaccharide molecule comprise an anionic group such as carboxylate, or the complementary acid or salt of such an anionic group. It

will be appreciated that some of the cellulose derivatives defined above will also be polyanionic polysaccharides. Preferred polyanionic polysaccharides include alginates, pectins, hyaluronic acid, oxidized starch and oxidized cellulose derivatives as described above. Most preferably, the polyanionic polysaccharide is an alginate. Naturally occurring sulfated polyanionic polysaccharides such a chondroitin sulfate are not included within the scope of the present invention, which relates only to synthetic (i.e. chemically sulfated) polysaccharide derivatives.

Oxidised regenerated cellulose (ORC) is a well known cellulose derivative that is prepared from cellulose as described in US-A-3122479. Oxidation of the primary hydroxyl groups of cellulose to carboxylate groups results in a polyanionic cellulose derivative, namely ORC. ORC is known and used in the biomedical field as a haemostatic agent, as described in US-A-2517772. It is commercially available in the form of a fabric under the registered trade mark Surgicel (Johnson & Johnson Medical, Inc.), and as a haemostatic wound treatment composition under the registered trade mark Interceed (Johnson & Johnson Medical, Inc.). It has been found that ORC is both haemostatic and fully bioabsorbable, and thus provides important advantages as a wound dressing material.

The term "alginate" used herein encompasses alginic acid, soluble salts thereof such as sodium alginate, and insoluble salts thereof such as calcium alginate. Preferably, the alginate is calcium alginate, which is available for use in wound dressings under the Registered Trade Marks Instat (Johnson & Johnson Medical, Inc.) and Kaltostat (E.R. Squibb & Sons, Inc.). Preferably, the alginate has a molecular weight in the range 50,000 to 400,000 and a mannuronic acid/guluronic acid ratio of about 0.45.

The sulfated polysaccharides according to the present

invention differ from the non-sulfated base materials in that at least some of the hydroxyl groups on the saccharide residues of the polysaccharides have been converted to sulfate groups. Preferably, an average of at least 0.1 hydroxyl groups on each saccharide residue have been converted to sulfate groups. More preferably, at least 1 hydroxyl group, on average, on each saccharide residue has been converted to sulfate groups, and most preferably from 3 to 4 hydroxyl groups on each saccharide residue have been so converted.

The sulfated polysaccharides can exist in the free acid form, but will more normally be used in the form of a salt or hydrate. The salt may be a salt with an alkali metal cation such as sodium or potassium, or may be a salt formed with polyvalent ions such as calcium or aluminum (by analogy with sucralfate), or may be a salt formed with complex cations such as ammonium or alkylammonium. Of course, the sulfated polysaccharide or its soluble salts may be stored or used in the form of a buffered aqueous solution. In any case, the term "sulfated polysaccharide" in the present specification encompasses all such salts and hydrates.

The sulfated polysaccharides according to the present invention can be made with substantially any molecular weight from 500 to 5,000,000, depending on the molecular weight of the starting material. Low molecular weight sulfated polysaccharides generally have alkali metal salts that are soluble in water, and accordingly such materials having molecular weights in the range 1,000 - 50,000 are preferred for some applications. These materials preferably have solubility of at least 10g/l, more preferably at least 25g/l in water at pH7.

When an insoluble sulfated material is desired, the base polysaccharide preferably has an average molecular weight in the range 50,000 - 250,000, more preferably 50,000 - 150,000. Such sulfated polysaccharides and/or their salts

are usually substantially insoluble in water. For example, insoluble sulfated ORC can be prepared from commercially available fibrous ORC in the form of a woven, non-woven or knitted fabric, thereby providing a sulfated ORC in the form of a fabric.

The present invention also provides a pharmaceutical composition comprising a sulfated cellulose derivative and/or a sulfated anionic polysaccharide according to the present invention. The term "pharmaceutical composition" includes wound treatment compositions and wound dressings and implants, as well as topical, oral or parenteral medicinal compositions.

It has been found, surprisingly, that the sulfated polysaccharides according to the present invention have an exceptional ability to bind to matrix metalloproteinases (MMP's). Such matrix matalloproteinases are implicated in a number of medical conditions, including chronic wounds such as decubitis ulcers. This is because the balance between matrix deposition and tissue turnover, which in turn may depend on the balance between proteolytic enzymes and their inhibitors, is fundamental to wound healing and to certain other medical conditions. Chronic wound fluids have been shown to contain elevated levels of MMP2 (Gelatinase A) and MMP9 (Gelatinase B).

Accordingly, the present invention further provides the use of a sulfated polysaccharide according to the present invention for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase. Preferably, the medical condition is a wound, especially a chronic wound or ulcer.

This aspect of the invention further encompasses a method for the treatment of a chronic wound such as a venous ulcer or a decubitis ulcer in a mammal by the topical or systemic administration of a pharmaceutical composition

containing the sulfated polysaccharide according to the present invention, preferably together with conventional pharmaceutical excipients. Preferably, the sulfated oligosaccharide is applied topically as or in a wound dressing composition. Preferably, the sulfated polysaccharide is applied in an ointment containing 0.1%-10% w/w of the sulfated polysaccharide together with conventional excipients such as water and a gelling agent.

It has also been found, surprisingly, that the sulfated polysaccharides according to the present invention has anticoagulant properties. Such properties are the exact opposite of the haemostatic properties well known for ORC itself. Accordingly, the present invention further provides the use of a sulfated polysaccharides according to the present invention for the preparation of an anticoagulant medicinal composition.

This aspect of the invention further comprises a method for the preparation of blood coagulation in a mammal by the topical or systemic administration of a pharmaceutical composition containing the sulfated polysaccharide according to the invention.

The pharmaceutical compositions according to the present invention may further comprise conventional pharmaceutical excipients and/or other active agents. They may be adapted for topical, oral or parenteral administration.

The sulfated anionic polysaccharides according to the present invention can be prepared by sulfation of a cellulose derivative or a polyanionic polysaccharide with sulfur trioxide-tertiary amine complexes or sulfur trioxide-pyridine complexes in a suitable inert polar solvent, such as N-methyl pyrrolidone. The degree of sulfation can be controlled by adjusting the molar ratio of polysaccharide to sulfur trioxide, since the reaction is essentially

quantitative. The extend of sulfation can be monitored by nmr and elemental analysis, and in particular by Infrared spectroscopy.

If a low molecular weight, soluble, sulfated polysaccharide derivative is desired, then the sulfation is preferably carried out on a low molecular weight, soluble oligosaccharide. For example, the sulfation can be carried out on ORC oligosaccharides obtained as described in the present applicant's co-pending United Kingdom patent application no. 9613683.3 entitled "Oxidised Oligosaccharides". Briefly, the ORC oligosaccharides are prepared by treating commercially available ORC with 6M sodium hydroxide solution at 37°C for 45 minutes, followed by filtration. The filtrate containing the ORC oligosaccharides in solution is subjected to neutralisation and dialysis to remove fragments and impurities having molecular weight below 1000, and is then freeze-dried.

Specific embodiments of the invention will now be described further, by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows the measured amount of MMP binding for a sulfated ORC derivative according to the present invention, compared with ORC itself and also compared with a collagen control; and

Figure 2 shows the measured amount of MMP2 and MMP9 binding for sulfated alginate/collagen sponges compared with a pure collagen sponge and non-sulfated alginate/collagen sponges.

Example 1

Milled ORC (Interceed®, 10g) was activated by slurrying in 100ml deionised water with stirring for 40 minutes at 40°C. The slurry was filtered using a Buchner apparatus, and the resultant cake was resuspended in 100ml glacial

acetic acid and stirred for a further 30 minutes at 4°C to remove any excess water. The slurry was again filtered and washed extensively with N-methyl pyrrolidone (NMP) until all the acetic acid was removed.

Pyridine sulfur trioxide (40g) was dissolved in 40ml N-methyl pyrrolidone, cooled to 4°C, and slowly added to the activated ORC. The slurry was stirred at 4°C for 2 hours, and the pH was then adjusted to 5.6 by the careful addition of cold 2M NaOH. The neutralised slurry was poured into three volumes of methanol and the precipitate collected. The sulfated material was washed with several portions of methanol to remove the N-methyl pyrrolidone before being washed with distilled water to remove unbound sodium sulfate. The material was frozen and freeze-dried.

FT-IR analysis of the material revealed that the all of the primary hydroxyl groups and one or more of the secondary hydroxyl groups had been sulfated. The product is the insoluble sodium salt of the sulfated ORC.

Example 2

The procedure of Example 1 was repeated on a 10g sample of Interceed® ORC fabric, resulting in a sulfated ORC fabric product.

Example 3

A composite material consisting of a complex between the sulfated ORC according to the present invention and collagen was prepared as follows.

Sulfated ORC fibres (0.875g) prepared as in Example 1 were suspended in 10ml distilled 0.06M acetic acid and stirred until fully dispersed. Bovine collagen, which was fully limed, freeze-dried and milled to 1mm fibers (1.625g) was slurried in 250ml 0.05M acetic acid and homogenised for 15 seconds on a Waring Blendor. The suspension of sulfated

ORC was added to the collagen slurry, and the mixture was homogenised for a further 15 seconds. The slurry was cross-linked by the addition of 0.0325ml of HMDI, followed by homogenisation for 2 x 15 seconds. The slurry was degassed under vacuum, poured into 9cm by 9cm petri dishes, frozen and freeze-dried. The resultant sponges were sterilised with gamma-irradiation.

Example 4

A sulfated calcium alginate was prepared from Alginic acid supplied by Sigma Chemical Company (A7003). A sample of this material (10g) was sulfated as described in Example 1.

Example 5

A composite material consisting of a complex between the sulfated alginate obtained in Example 4 and collagen was prepared by the procedure described in Example 3, using milled sulfated alginate fibers in place of the sulfated ORC fibers.

Procedure 1: Effect of Sulfation on Matrix Metalloproteinase Binding by ORC

The effect of sulfation of ORC on matrix metalloproteinse (MMP) binding was assessed as follows.

Sulfated ORC was prepared by the procedure described in Example 2. An ORC-free collagen sponge was prepared for comparison purposes. A sample of Surgicel® ORC fabric was also prepared for comparison.

Briefly 50mg of each material was placed in a 15ml plastic beaker containing 2.5ml of an acute wound fluid diluted to 1:50 in a proteolysis buffer (50mM tris/HCL pH7.8, 50mM $CaCl_2$, 0.5M NaCl) and incubated at 37°C on a shaking water bath for 3 hours. Acute wound fluid contains various proteinases, including matrix metalloproteinases and many of these enzymes will preferentially bind to various

dressing materials. The excess fluid absorbed by each material was mechanically expressed using a metal spatula and discarded. The remaining dressings were placed into pre-packed 2ml syringes (each syringe contained 0.5ml volume of 2.5mm glass beads). 4ml of proteolysis buffer was forced through the syringe in 1ml aliquots which were discarded. At this washing stage all of the unbound proteinases and proteinaseses which were only weakly bound to the dressing material had been removed from the dressing leaving the more tightly bound forms. The buffer rinsed dressing were then removed to another 15ml plastic beaker. 1ml of non-denaturing sample buffer (6.3ml 0.05M tris/HCL pH6.8, 2.5ml glycerol, 0.5g SDS, 16.2ml water and bromophenol blue) was added to each sample which were placed on an orbital shakier at setting six for 2 hours. The sample buffer detaches the tightly bound proteinases from the materials which are then present in the sample buffer itself. After this time 20 microliters of sample buffer was taken from each container and subjected to gelatin substrate SDS-polyacrylamide gel electrophoresis (zymography) as described by Heussen C. and Dowdle E.B. in Anal. Biochem. 102:196-202 (1980).

The area of the individual zones of clearance on the gels, which are due to proteinase activity, were accurately measured by the Optilab system. This was achieved by repeating each binding experiment (n=3) and analysing the results statistically by the Students T test, where $P \leq 0.05$. Analysis was against controls of pure collagen.

The results shown in Figure 1 demonstrate a surprising synergistic improvement in MMP binding for the sulfated ORC. In Figure 1, Pro2 and Pro9 are the proenzyme forms of MMP2 and MMP9. Both MMP2 and MMP9 can exist as active an proenzyme forms. The molecular weight of the proMMP9 or pro9 is 92kDa. MMP activation is a two step process, a conformational change occurs in the proenzyme, followed by removal of the propeptide by a series of autocatalytic changes. The molecular weight of the resultant active forms

being 86kDa for ActMMP9 and 66kDa for ActMMP2, sometimes called ACT9 and ACT2 for short.

Procedure 2: Effect of Sulfation of Alginate on Matrix Metalloproteinase Binding by Collagen-Alginate Complexes:

Procedure 1 was repeated on the following samples: a pure collagen sponge (comparative experiment), collagen/calcium alginate sponges containing 5% and 20% w/w of alginate (comparative experiments identified as 5% ALG and 20% ALG), and collagen/sulfated calcium alginate sponges containing 5%, 10% and 20% w/w of sulfated calcium alginate, prepared as described in Example 5 and identified as 5% SALG, 10% SALG and 20% SALG.

The results are shown in Figure 2. It can be seen that the collagen/sulfated alginate sponges exhibit much stronger binding to both MMP2 (Gelatinase A) and MMP9 (Gelatinase B) than the comparative sponges.

Procedure 3: Effect of Sulfation of ORC on Blood Coagulation

The effect of sulfation on the coagulant properties of ORC is studied as follows.

Fresh rat's blood (1ml) is measured into 7ml sterile tubes containing 0(control), 0.1g or 1.0g of sulfated ORC fibers prepared as in Example 1. The tubes are gently agitated for 1 minute, and then at 20 second intervals. The length of time taken for the blood to clot is recorded. The results are as follows:

Weight of Sulfated ORC Fibers (g)	Time to Clot(s)	
	With Sulfated ORC	Control
0.1	510	380
1.0	735	490

This demonstrates that the sulfated ORC fibers have an anticoagulant effect. This result is unexpected in view of

the known haemostatic properties of ORC itself.

Procedure 4: Effect of Sulfated ORC on Haemostatis

The effect of sulfated ORC on haemostasis was investigated using the swine spleen model, as follows:

Recently weaned, female, cross-bred swine, in the approximate weight range of 22-45kg, were anaesthetised with Isoflurane (Aerrane®). A surgical plan of anaesthesia was achieved and demonstrated by a null response to a noxious stimulus. While under anaesthesia, physiological parameters such as temperature, pulse and respiration were monitored and documented.

Animals were placed in dorsal recumbrance with all limbs secured. The abdominal cavity was opened along the midline. The spleen was exteriorized. Haemostasis incision wounds were made using a scalpel on the surface of the spleen. Wound lengths were controlled and ranged from 0.5 to 2.0cm. Wound depth was controlled and ranged from approximately 1.5 to 3.0mm deep. The depth of each wound was kept constant by marking the scalpel blade at the appropriate depth. The length of the incision was controlled by using a suitable template which had been clearly marked for the appropriate incision length. The first wound at the distal end of the spleen served as a negative control and was permitted to bleed for twelve minutes to demonstrate the bleeding potential of an untreated wound. The second wound was made approximately 1.0cm proximal to the first incision. This and subsequent incisions were used as test incisions. A final incision was used as a termination negative control to demonstrate that the bleeding potential of an untreated wound did not change.

After incisions were created, a stop-watch was started and collagen sponge (Instat) or collagen/sulfated ORC sponge prepared as in Example 3 were quickly applied to the wounds. Gentle pressure was applied to the top surface or the gauze

for 2 minutes and then the pressure was released. This procedure was repeated at 30 second intervals until the haemorrhage was controlled. Control of the haemorrhage (haemostasis) was defined as no renewed bleeding for 30 seconds. The time of the last release of pressure was the time to achieve haemostasis. The order in which the test or control articles were placed on the wounds was assigned by computerised randomisation.

Animals were euthanized by I.V. injection with a commercially available solution or other suitable means before recovering from anaesthesia.

These studies showed that the presence of sulfated ORC retarded haemostasis relative to a pure collagen sponge.

The above described specific embodiments of the present invention are intended solely for the purpose of illustration. Many other embodiments falling within the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. A sulfated polysaccharide selected from the group consisting of sulfated cellulose derivatives and sulfated polyanionic polysaccharides.

2. A sulfated polysaccharide according to claim 1, selected from the group consisting of sulfated hydroxyethyl cellulose, sulfated carboxymethyl cellulose and sulfated oxidized regenerated cellulose.

3. A sulfated polysaccharide according to claim 1 which is sulfated oxidized regenerated cellulose.

4. A sulfated polysaccharide according to claim 1, selected from the group consisting of sulfated alginates, sulfated pectins and sulfated hyaluronic acid.

5. A sulfated polysaccharide according to claim 1 which is a sulfated alginate.

6. A sulfated polysaccharide according to any preceding claim, comprising an average of at least 0.1 sulfate groups for each saccharide residue of the polysaccharide.

7. A sulfated polysaccharide according to claim 6, comprising an average of at least 1 sulfate group for each saccharide residue of the polysaccharide.

8. A sulfated polysaccharide according to any preceding claim having an average molecular weight in the range 25,000 - 250,000.

9. A sulfated polysaccharide according to any preceding claim in the form of a woven, non-woven or knitted fabric.

10. A sulfated polysaccharide according to any preceding claim in the form of a solid complex with collagen.

11. A sulfated polysaccharide according to any of claims 1 to 7 which is soluble in water to an extent of at least 10g/l at 25°C.

12. A pharmaceutical composition comprising a sulfated polysaccharide according to any of claims 1 to 12.

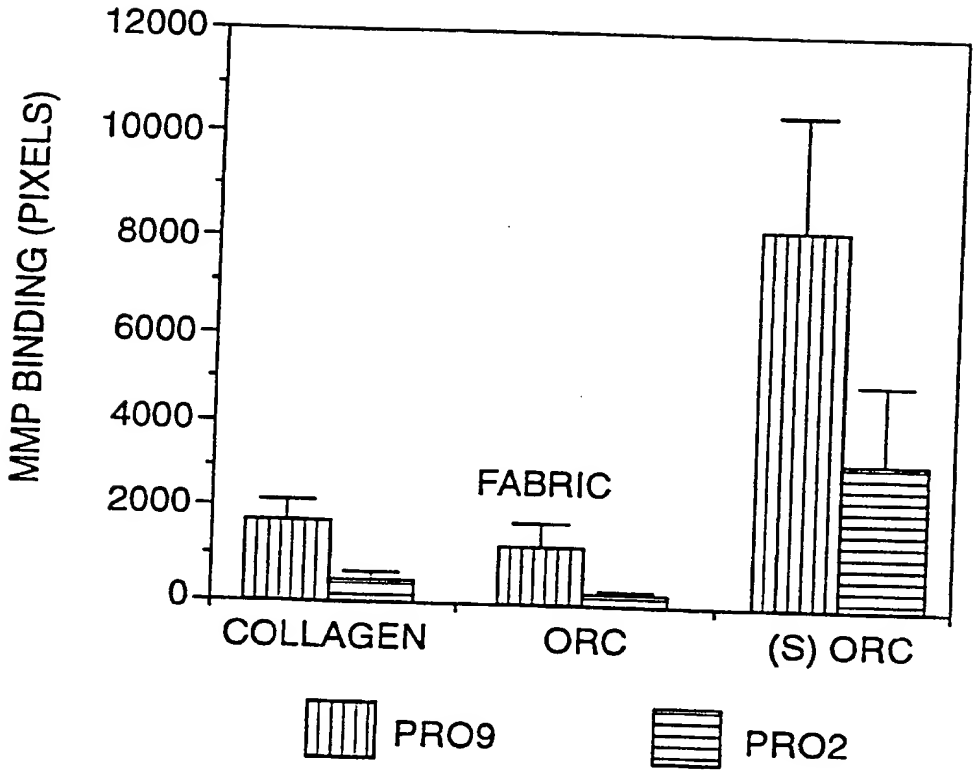
13. A pharmaceutical composition according to claim 12 in the form of a wound dressing material or a soft tissue implant.

14. Use of sulfated polysaccharide according to any of claims 1 to 12 for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase.

15. Use according to claim 14, wherein the medical condition is a chronic wound.

16. Use of a sulfated polysaccharide according to any of claims 1 to 12 for the preparation of a pharmaceutical composition for the prevention or reduction of blood coagulation.

FIG. 1


12000
10000
8000
6000
4000
2000
0
MMP BINDING (PIXELS)
FABRIC
COLLAGEN
ORC
(S) ORC
PRO9
PRO2

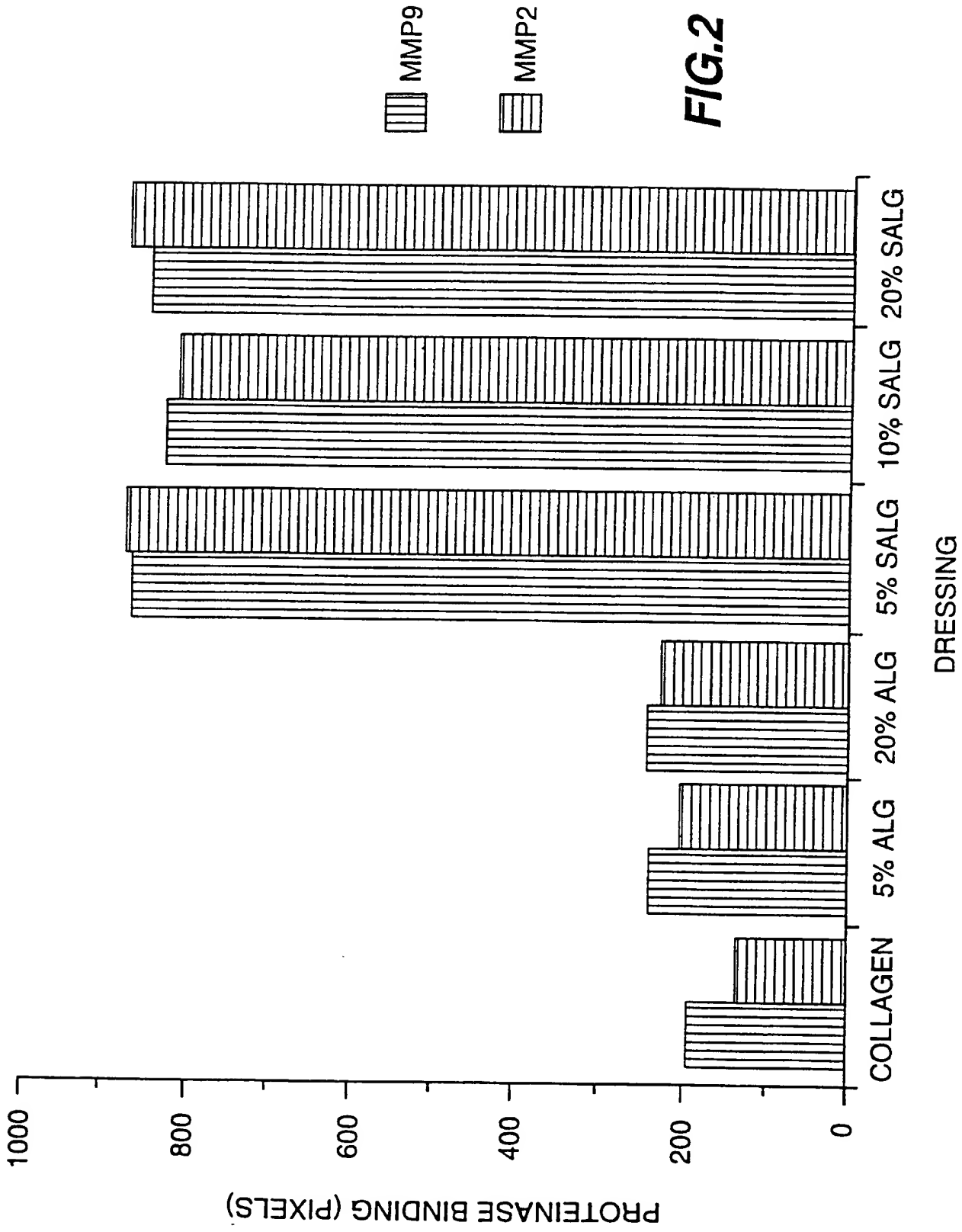

MMP9
MMP2
PROTEINASE BINDING (PIXELS)
1000
800
600
400
200
0
COLLAGEN
5% ALG
20% ALG
5% SALG
10% SALG
20% SALG
DRESSING

FIG.2

INTERNATIONAL SEARCH REPORT

Inte[redacted]pplication No
PCT/GB 97/02477

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B15/00 C08B37/04 A61K31/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RICHARD G. SCHWEIGER ET AL.: "Polysaccharide Sulfates." CARBOHYDRATE RESEARCH., vol. 21, 1972, AMSTERDAM NL, pages 275-281, XP002049669 see the whole document ---	1-16
X	KURT MAURER ET AL.: "Die Synthese heparinwirksamer Verbindungen aus carboxylierter Cellulose." CHEMISCHE BERICHTE., vol. 80, no. 1, 1947, WEINHEIM DE, pages 179-187, XP002049670 see page 181 ---	1-16
X	FR 1 056 772 A (HENRI MORREN) 2 March 1954 see page 1, column 2, last paragraph - page 2, column 1, paragraph SECOND --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 1997	14/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lensen, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int[illegible] Application No
PCT/GB 97/02477

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 140 596 A (JOHNSON & JOHNSON) 8 May 1985 see page 4, line 21 - page 5, line 4	1-16
A	US 3 709 877 A (DEGER TUNE) 9 January 1973	
A	DE 25 46 699 A (SUMITOMO CHEMICAL) 29 April 1976	
A	US 4 879 282 A (SALIBA JR.) 7 November 1989	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/GB 97/02477

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 1056772 A	02-03-54	NONE	
EP 140596 A	08-05-85	GB 2148901 A	05-06-85
		AU 573626 B	16-06-88
		AU 3380384 A	18-04-85
		CA 1234102 A	15-03-88
		DE 3472263 A	28-07-88
		US 4614794 A	30-09-86
US 3709877 A	09-01-73	AR 192687 A	28-02-73
		AU 4990672 A	13-06-74
		CA 965783 A	08-04-75
		GB 1407064 A	24-09-75
		ZA 7208413 A	26-06-74
DE 2546699 A	29-04-76	JP 51052484 A	10-05-76
		FR 2287911 A	14-05-76
US 4879282 A	07-11-89	AU 1573588 A	10-10-88
		CA 1315683 A	06-04-93
		WO 8806840 A	22-09-88
		US 5037810 A	06-08-91

CLAIMS

1. A ~~sulfated~~ synthetic polysaccharide selected from the group consisting of sulfated cellulose derivatives and sulfated polyanionic polysaccharides.

2. A sulfated polysaccharide according to claim 1, selected from the group consisting of sulfated hydroxyethyl cellulose, sulfated carboxymethyl cellulose and sulfated oxidized regenerated cellulose.

3. A sulfated polysaccharide according to claim 1 which is sulfated oxidized regenerated cellulose.

4. A sulfated polysaccharide according to claim 1, selected from the group consisting of sulfated alginates, sulfated pectins and sulfated hyaluronic acid.

5. A sulfated polysaccharide according to claim 1 which is a sulfated alginate.

6. A sulfated polysaccharide according to any preceding claim, comprising an average of at least 0.1 sulfate groups for each saccharide residue of the polysaccharide.

7. A sulfated polysaccharide according to claim 6, comprising an average of at least 1 sulfate group for each saccharide residue of the polysaccharide.

8. A sulfated polysaccharide according to ~~any preceding~~ claim having an average molecular weight in the range 25,000 - 250,000.

9. A sulfated polysaccharide according to ~~any preceding~~ claim in the form of a woven, non-woven or knitted fabric.

10. A sulfated polysaccharide according to ~~any preceding~~ claim in the form of a solid complex with collagen.

Subst B4

11. A sulfated polysaccharide according to any of claims 1 to 7 which is soluble in water to an extent of at least 10g/l at 25°C.

12. A pharmaceutical composition comprising a sulfated polysaccharide according to any of claims 1 to 12.

13. A pharmaceutical composition according to claim 12 in the form of a wound dressing material or a soft tissue implant.

Subst A'

14. Use of sulfated polysaccharide according to any of claims 1 to 12 for the preparation of a composition for the treatment of medical conditions mediated by a matrix metalloproteinase.

15. Use according to claim 14, wherein the medical condition is a chronic wound.

16. Use of a sulfated polysaccharide according to any of claims 1 to 12 for the preparation of a pharmaceutical composition for the prevention or reduction of blood coagulation.